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Sub-chronic Toxicity Assessment of Local Textile ‘Adire and Kampala’ (Tie and Dye) Effluents on Mice (*Mus musculus*)

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ABSTRACT

‘Adire and Kampala’ (Tie and dye) have been produced in Itoku, Abeokuta, Ogun state-Nigeria for over a century. Large quantity of water has been involved and effluents have been discharged into surrounding sources of water without adequate treatment. The evaluation of the sub-chronic toxicity of the Adire and Kampala textile effluents is done by investigating its effect on the weights, histopathologic and haematologic indices in normal albino mice. Normal albino mice of either sex weighing between 23 and 37 g were divided into five groups of 10 animals each. Four concentrations were orally administered 1, 5, 25 and 50% while control animals received distilled water over 28 days. Animals were weighed weekly and sacrificed after day 28. Organs were harvested, weighed and subjected to histopathologic assessment. Blood samples were used for haematological studies. The 30 and 50% mortality was observed in the groups administered 25 and 50% concentration, respectively and several signs of toxicity were observed in other groups. Decreased organs and body weight, WBC, PCV and Hb significantly different from control were observed. Histopathologic studies showed remarkable distortion in the histo-architecture of the visceral organs, like alveoli disruption, spleen pigmentation, tubular necrosis in kidney and hemorrhage in the heart. The mortality observed, decreased haematologic indices, internal organs, body weight and gross distortion in the histo-architecture of the visceral organs are indications that the ‘Adire and Kampala’ textile effluents contain substances that are toxic to the animal physiology. These substances which may have seeped into surrounding rivers and wells in Abeokuta town can cause severe health malaise in the inhabitants of such area.

Key words: Textile effluents, pollution, toxicity, haematology, histopathology

INTRODUCTION

Textile industry is reported to be one of the most common and essential industrial sectors in the world and plays a prominent role in nation's economy (Eremektar *et al.*, 2007).

Abeokuta is noted for the production of local textiles which are popularly known as Adire and Kampala and are regarded as the best known pattern dyed cloth in Nigeria for over a century ago and the local textiles are made in the form of tying and dyeing. The major production site of this local textile is Itoku situated in Abeokuta south local Government area of Ogun state (Salami, 2001). The ‘Adire and Kampala’ (Tie and dye) business in Abeokuta, provides substantial contribution to the economy in the form of income, employment and possibly foreign exchange generation.

Rivers and wells (a device, hole or shaft usually vertical, excavated in the earth for bringing ground water to the surface) are important multi-usage systems which serve as sources of drinking water, irrigation, fishery and energy production (Hacioglu and Dulger, 2009). Almost all the freshwater bodies are being polluted by expanding human population and in consequence, industrialization, intensive agricultural practices and discharge of massive amount of waste water etc., which result in deterioration of water quality and consequently harmful for human consumption. There is evidence to suggest that these local textiles production utilize considerable quantity of water and discharge the effluents without adequate treatment. The discharge of effluents has caused severe pollution of both the surface, shallow wells and ground water in the region.

Toxicity evaluation has been alluded an important parameter in wastewater quality monitoring in order to protect aquatic organisms and human health (Liber *et al.*, 2005).

Against these backdrops, sub chronic toxicity assay exposing mice to dye effluent (50 and 75%) of Adire factory was studied on various haematological parameters (WBC, Hb%, PCV, MCHC, N, L, E, M) and histology of accessory organs examined on Swiss albino mice *Mus musculus*.

MATERIALS AND METHODS

Study area: The study area Abeokuta metropolis is a millennium city and the state headquarter of Ogun state in Southwestern Nigeria. It falls within latitudes 7° 6'N to 7° 13'N and longitudes 3° 16'E to 3° 25'E. Abeokuta is situated about 70 km north of Lagos and together with its environ has an area coverage of about 212 km².

Textile effluents: Concentrated textile effluents were collected from the site of textile production at Itoku situated in Abeokuta south local Government area of Ogun state. Effluents were prepared into 1, 5, 25 and 50%.

Animals: Normal albino mice of either sex, weighing between 23 and 37 g, were purchased from the Nigerian Institute of Medical Research, Yaba and Lagos, Nigeria. Animals were housed in aerated metal cages in the animal house of the Redeemer's University, acclimatized for 1 week before experiments and fed with standard rat chow manufactured by Ladokun Feeds Limited, Ibadan, Nigeria and water ad libitum. Mice were maintained under standard environmental conditions (Mbagwu *et al.*, 2007) throughout the experimental period.

Sub-chronic oral toxicity study of textile effluents in mice for 28 days

Study design: Animals divided into 5 groups of 10 mice each were treated with the different concentrations for 28 days by oral gavage using a curved blunt tipped stainless steel feeding needle. Group 1 (control) was treated with distilled water 10 mL kg⁻¹ while groups 2, 3 and 4 received effluents at concentration of 1, 5, 25 and 50%, respectively. The weights of the animals were monitored and recorded weekly for 4 weeks after which animals were sacrificed. The organs and tissues were then collected, weighed and processed on day 28 for relevant assays and histopathologic analysis.

Mortality and clinical signs: During the administration period of 28 days, animals were observed for general appearance, mortality and clinical signs.

Hematology, relative organ weight and necropsy: The mice were fasted for 16-19 h on the autopsy day and anesthetized with ethyl ether. All the animals were then euthanized by

exsanguination and blood samples collected from the abdominal aorta into EDTA vials for routine hematological investigation. The spleen, heart, liver, kidneys and lungs were harvested and weighed to determine the absolute organ weight. The relative organ weight of each animal was then calculated based on body weight measured on the day of sacrifice as follows:

$$\text{Relative organ weight (g)} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

The organs were thereafter preserved in 10% buffered formalin for histopathologic examinations. The tissue biopsies were dehydrated and embedded in paraffin, cut into 4-5 μm sections with rotary microtome (LEICA RM 2235 Rotary Microtome) and stained with hematoxylin eosin for photomicroscopic examination.

RESULTS

Clinical signs and mortality: All the mice administered with the effluents at all concentrations showed significant weight reduction with the highest emaciation observed in 5, 25 and 50%, respectively (Table 1). A 30 and 50% mortality was observed in the 25 and 50% concentration, respectively.

Relative organ weight: Relative organ weight represents the ratio of organ weight to overall body weight (Table 2). There was significant reduction in the weight of the heart and lungs of animals administered 25 and 50%, respectively ($p > 0.05$).

Haematologic values: The haematological indices showed that the effect of the effluent was remarkable in all the parameters observed. There were significant reduction in the WBC and Hb. The PCV decreased for 33.00 ± 0.39 to 13.50 ± 0.99 and MCHC decreased from 33.30 ± 0.10 to 16.70 ± 1.10 (Table 3) concentration-dependently from 1 to 50%, respectively. A decreased N, L, E and M% values were observed at all exposure periods when compared to control group (Table 3).

Table 1: Effect of effluent on body weight of mice for four weeks

Treatment	Week			
	1	2	3	4
Control	23.73 $\pm$ 0.30	25.70 $\pm$ 0.32	26.90 $\pm$ 0.35	28.47 $\pm$ 0.36
1%	32.73 $\pm$ 0.33 ^b	32.13 $\pm$ 0.40 ^b	31.52 $\pm$ 0.40 ^b	30.10 $\pm$ 0.40 ^b
5%	34.34 $\pm$ 0.32 ^c	33.43 $\pm$ 0.35 ^c	32.23 $\pm$ 0.40 ^c	30.99 $\pm$ 0.40 ^c
25%	36.80 $\pm$ 0.44 ^c	35.90 $\pm$ 0.45 ^c	34.52 $\pm$ 0.40 ^c	34.14 $\pm$ 0.40 ^c
50%	34.34 $\pm$ 0.32 ^c	33.43 $\pm$ 0.40 ^c	32.23 $\pm$ 0.40 ^c	22.94 $\pm$ 0.56 ^c

Values are Mean $\pm$ SEM (N=10/group), ^bcSignificantly different from controls at $p < 0.01$ and $p < 0.001$

Table 2: Effect of effluent on relative organ weight of mice for four weeks

Treatment	Liver	Right kidney	Left kidney	Heart	Lung	Spleen
Control	1.57 $\pm$ 0.09	0.19 $\pm$ 0.04	0.21 $\pm$ 0.05	0.14 $\pm$ 0.03	0.29 $\pm$ 0.04	0.30 $\pm$ 0.08
1%	1.83 $\pm$ 0.09	0.19 $\pm$ 0.04	0.19 $\pm$ 0.05	0.14 $\pm$ 0.03	0.30 $\pm$ 0.04	0.25 $\pm$ 0.09
5%	1.70 $\pm$ 0.16	0.20 $\pm$ 0.04	0.20 $\pm$ 0.04	0.20 $\pm$ 0.04	0.30 $\pm$ 0.05	0.20 $\pm$ 0.07
25%	1.90 $\pm$ 0.11	0.20 $\pm$ 0.06	0.23 $\pm$ 0.06	0.20 $\pm$ 0.03 ^b	0.30 $\pm$ 0.05	0.30 $\pm$ 0.03
50%	1.30 $\pm$ 0.12	0.23 $\pm$ 0.04	0.22 $\pm$ 0.00	0.14 $\pm$ 0.03	0.22 $\pm$ 0.06 ^a	0.24 $\pm$ 0.10

Values are Mean $\pm$ SEM (N=10/group), ^aSignificantly different from controls at $p < 0.05$

Table 3: Haematological values of mice orally administered with effluent for four weeks

Dose	PCV (%)	WBC $\times 10^9$ L ⁻¹	Hb (g dL ⁻¹)	MCHC (g dL ⁻¹)	N (%)	L (%)	E (%)	M (%)
Control	33.75 $\pm$ 0.53	5600 $\pm$ 8.90	12.25 $\pm$ 0.24	33.38 $\pm$ 0.10	6.50 $\pm$ 0.60	32.25 $\pm$ 0.65	0.25 $\pm$ 0.20	0.00 $\pm$ 0.00
1%	33.00 $\pm$ 0.39	5275 $\pm$ 11.90	11.00 $\pm$ 0.23	33.30 $\pm$ 0.10	64.00 $\pm$ 0.63	35.00 $\pm$ 0.60	0.75 $\pm$ 0.20	0.75 $\pm$ 0.20
5%	30.00 $\pm$ 0.51	5025 $\pm$ 10.96	22.25 $\pm$ 0.31 ^b	33.30 $\pm$ 0.00	66.30 $\pm$ 0.47	33.00 $\pm$ 0.50	0.50 $\pm$ 0.20	0.75 $\pm$ 0.24
25%	28.75 $\pm$ 0.70	4425 $\pm$ 11.90 ^b	9.90 $\pm$ 0.17 ^c	33.20 $\pm$ 0.10	60.80 $\pm$ 0.41	39.25 $\pm$ 0.47	0.25 $\pm$ 0.20	0.00 $\pm$ 0.00
50%	13.50 $\pm$ 0.99	1925 $\pm$ 12.00	4.50 $\pm$ 0.57 ^c	16.70 $\pm$ 1.10	28.00 $\pm$ 1.40	21.00 $\pm$ 1.20	0.25 $\pm$ 0.20	0.75 $\pm$ 0.24

Values are mean $\pm$ SEM (N=10/group), ^{b,c}Significantly different from controls at $p < 0.01$ and $p < 0.001$

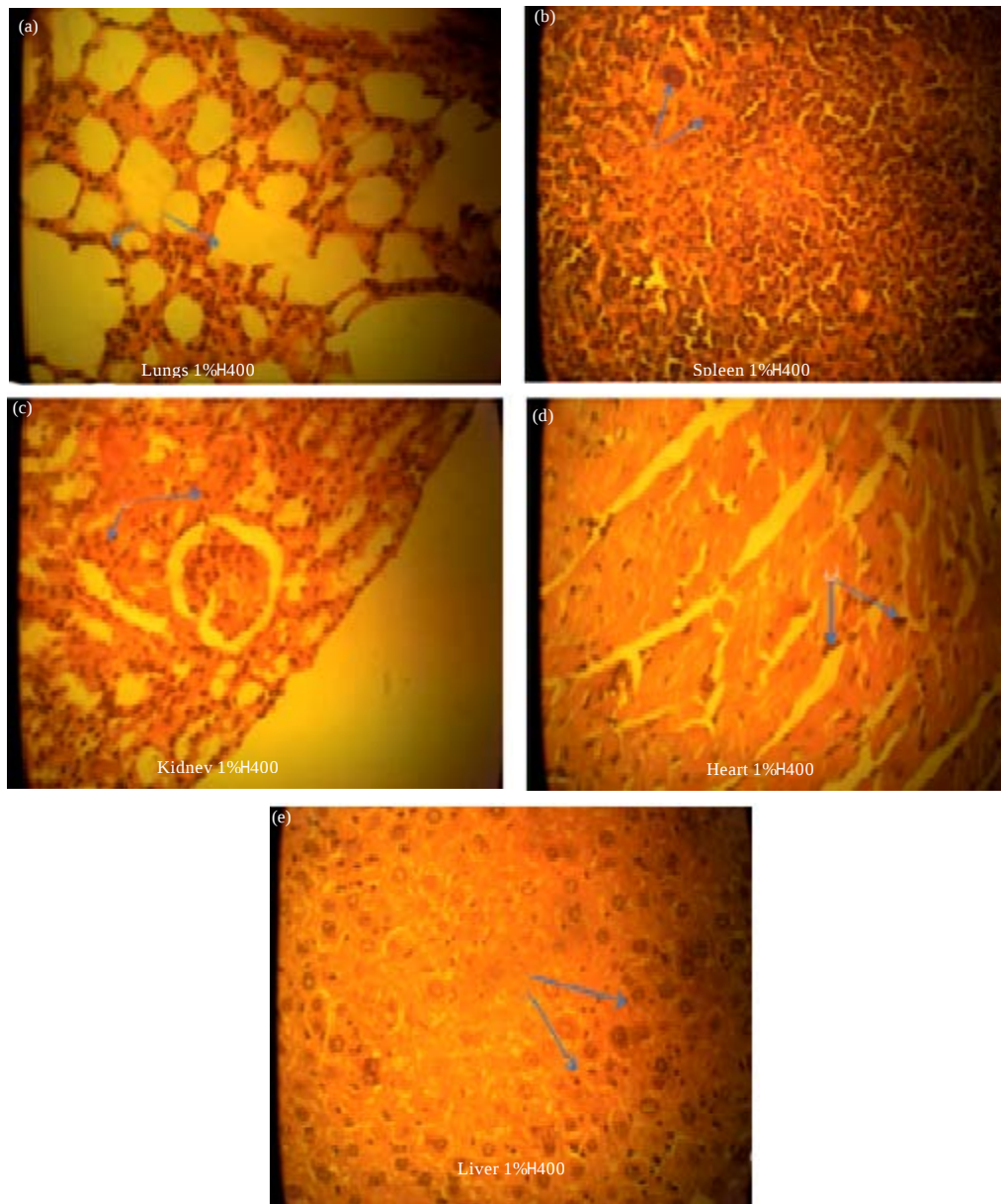


Fig. 1(a-e): (a) Marked distortion of the alveoli wall leading to varying alveoli spaces, (b) Pigment deposit in spleen, (c) Acute tubular necrosis in kidney and (d) Hemorrhage in the heart

Histology: There were various distortions observed in the histopath of the internal organs. There was marked distortion in the alveoli of the lungs leading to varying alveoli spaces, deposits of pigments were observed in the spleen, tubular necrosis in the kidney and haemorrhage in the heart (Fig. 1).

DISCUSSION

This study was designed to evaluate the sub-chronic toxicity of 'Adire' (Tie and dye) textile effluents in normal albino Swiss mice (*Mus musculus*). A 30 and 50% mortality observed in the 25 and 50% concentration showed that the effluents contained deleterious constituents that may be harmful to organisms that utilize such water. Decreases in weight and relative organ weight also suggest that the effluents possess substances that may affect the body physiology of organisms.

Haematopoietic indices have been reported to be very sensitive to toxic compounds and serve as important index of physiologic and pathologic status for both animals and humans (Rosidah *et al.*, 2009). The significant decreases in WBC, PCV, MCHC, Hb and other haematologic profile is an indication that the effluent may distort the production of the blood cells in the haematopoietic tissues. Sharma *et al.* (2007) reported decrease in RBC, Hb% and PCV of rat treated with textile dye which may be ascribed to decline in their survival period when treated with dyes or toxic effects on haemopoietic cells in the bone marrow (Lee *et al.*, 1999). Significant decrease in haemoglobin content and packed cell volume was also observed in mice treated with fellon (Kumari *et al.*, 2013), sodium benzoate (Sinha and D'souza, 2008), fluoride (Choudhary *et al.*, 2008), distillery effluent (Varma and Pratap, 2006). The MCHC expresses the concentration of haemoglobin in the cytoplasm of the erythrocytes. MCV count and MCHC is dependent on the RBC count, Hb content and PCV value. Due to effluent toxicity, the bone marrow lacks the capacity to manufacture haemoglobin at the required rate, so the haemoglobin content of each cell diminished the MCHC value. Decreased PCV value indicates oxygen carrying capacity of blood and the degree of stress on animal health (Larsson *et al.*, 1985). Reduction in PCV values of rats exposed to dye effluent also indicates anaemia (Wepener and Vuren, 1992).

Normally the globin portion of haemoglobin is broken down into amino acids which return to the protein port while; porphyrin is metabolized and accredited as bile pigment. The iron released from breaking of haemoglobin is carried by transferrin either to bone marrow for production of new red blood cells or to the liver for storage in the form of ferritin. The synthesis of haemoglobin requires iron, which is generally supplied from the stored ferritin. Therefore, it seems that the dye may have prevented the iron supply for the synthesis for haemoglobin by inhibiting the absorption by developing erythrocytes, which resulted in the fall of haemoglobin content, therefore the rate of haemoglobin synthesis decreases during all stages of maturation of erythrocytes when the supply of iron was not sufficient.

In addition to being toxic, dye effluents also contain chemicals that are carcinogenic, mutagenic or teratogenic to various organisms (Novotny *et al.*, 2006; Mathur and Bhatnagar, 2007), however many dyes are made from known carcinogens like benzidine and are also known to accumulate, thus posing a serious threat (Baughman and Perenich, 1988). Many dyes are also known to get reduced to toxic substances inside living organisms (Weber and Wolfe, 1987).

Histopathologic examination showed remarkable distortion in the histo-architecture of the kidney, lung, spleen and heart, there was alteration in the alveoli spaces, deposits of pigment in the spleen, tubular necrosis in the kidney and hemorrhage in the heart.

CONCLUSION

The effluents from textile industry contain a variety of harmful chemicals. These chemicals severely affect the quality of water. Since quantitative measurement of these pollutants is not possible because they undergo chemical transformations in environment. Regular monitoring of water quality is therefore essential. From the findings, it was found that 'Adire' textile effluents has a deleterious effect upon the weight, blood profile, lungs, kidney, spleen and heart of mice, which can lead to many metabolic and physiological disorders. The present investigation may be a valuable step in the toxicity assessment of 'Adire' textile effluents in residents of the production area as it seeps into their wells and rivers.

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